Quantitative Determination of Phenols in Mainstream Smoke with Solid-Phase Microextraction–Gas Chromatography–Selected Ion Monitoring Mass Spectrometry

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Abstract

A method that uses solid-phase microextraction–gas chromatography–selected ion mode mass spectroscopy has been developed for the quantitative determination of phenolic compounds in cigarette smoke condensate. The concentrations of phenol, *o*-, *m*-, and *p*-cresol, 2-methoxyphenol, 2,6-dimethylphenol, 2,4-dimethylphenol, 2,5-dimethylphenol, 4-ethylphenol, 3-ethylphenol, 2,4,6-trimethylphenol, 4-methoxyphenol, 3-methoxyphenol, vanillin, 1-napthol, and 2-napthol present in Kentucky Reference 2R1F and five commercial cigarette brands are determined. Minimal sample preparation is required, and no organic solvents or derivatization reagents are necessary.

Introduction

Numerous methods have been reported for the quantitative determination of phenols in cigarette smoke condensate (CSC) (1–12). Although current methods provide very precise data, they involve extensive sample preparation that often includes isolation and derivatization.

Gas chromatographic (GC) separation followed by selected ion monitoring (SIM) mass spectrometry (MS) has been used for the quantitative analysis of the major phenolic compounds in cigarette smoke (1). The cited method relies upon conversion of the phenolics to their trimethylsilyloxy ethers. The recoveries, method precision, detection limits, and dynamic range indicate the method performs well. The less abundant phenolics have been evaluated to a lesser extent (2,3). Snook and coworkers (3) qualitatively identified over 60 phenolic acids in mainstream smoke by GC–MS. The only quantitative study of minor phenolics involves a difficult sample preparation (2). With ever increasing environmental concerns, the use of solvents and derivatization reagents is less desirable for chemical analysis.

Solid-phase microextraction (SPME) has provided outstanding results in the quantitative analysis of substituted benzenes (13), phenolics (14,15), polycyclic aromatic hydrocarbons (16), and polychlorinated biphenyls (16), and in qualitative flavor analysis (17,18). SPME is of special interest since it provides extremely low detection limits and exhibits linearity over a wide range of concentrations for the chemical classes that have been reported. Current SPME technology uses water as a solvent. This technique is attractive due to current environmental concerns and high costs associated with solvent disposal.

To date, SPME techniques have not been reported for the analysis of tobacco extracts or cigarette smoke. This methodology has excellent potential for determination of phenolics in mainstream smoke given its success in environmental analysis.

Experimental

Instrumentation

A Hewlett-Packard (HP) 5890 gas chromatograph (Palo Alto, CA) directly interfaced with an HP 5970B mass selective de-

Table I. Mass Spectral Detection Parameters

Phenolic compound	Retention time (min)	Ions monitored*
Phenol	15.99	**94**, 66
o-Cresol	18.61	**108**, 107, 79, 77
m- & *p*-Cresol	19.41	**108**, 107, 79, 77
2-Methoxyphenol	19.72	**124**, 94, 81
2,6-Dimethylphenol	20.28	**122**, 107, 77
2,4- & 2,5-Dimethylphenol	21.82	**122**, 107, 91, 77
2'-Hydroxyacetophenone	22.17	136, **121**, 93, 65
4-Ethylphenol	22.35	122, **107**, 94, 77
3-Ethylphenol	22.44	122, **107**, 94, 77
2,4,6-Trimethylphenol	23.45	136, **121**, 91, 77
4-Methoxyphenol	24.01	**124**, 94, 81, 66
3-Methoxyphenol	24.29	**124**, 94, 81, 66
Vanillin	29.43	**151**, 109, 81
1-Napthol	32.11	**144**, 115, 89, 72
2-Napthol	32.35	**144**, 115, 89, 72

*Ions used for quantitation are given in bold.

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tector was used to separate and analyze the phenolic components. The instrument was controlled by an HP G1030A DOS Chemstation using the standard quantitation programming. Injections were made at 275°C with a manual SPME syringe equipped with a polyacrylate SPME fiber (Supelco; Bellefonte, PA). Separation was accomplished with an HP DB-5MS column (30 m × 0.23-mm i.d. × 0.20-µm film thickness). The initial oven temperature was 35°C. It was held for 7 min and then increased to 240°C at a rate of 5°C/min. The GC–MS transfer line was maintained at 280°C. Mass spectrometric detection was made in the selected ion mode. Selected mass spectral ions monitored and used for quantitation are listed in Table I.

Calibration standards

Reagent-grade standards purchased from Aldrich Chemical (St. Louis, MO) and ChemService (Westchester, PA) were used. Standard solutions were prepared by weighing 100 mg each of phenol, *o*-cresol, *m*-cresol, 2-methoxyphenol, 2,6-dimethylphenol, 2,4-dimethylphenol, 3-ethylphenol, 2,4,6-trimethylphenol, vanillin, 1-napthol, and 2-napthol and diluting the compounds to 100 mL (approximately 1000 µg/mL) with HPLC-grade methanol. The standard solution (0.1, 0.5, 5, 10, 25, and 50 µL or 0.1, 0.5, 5, 10, 25, and 50 µg) and 30.5 µg of 2'-hydroxyacetophenone (ISTD) were added to 15 mL of a buffer solution (pH 2) prepared with 25 mL of 0.2M KCl and 6.5 mL of 0.2M HCl in 100 mL of water (14). A 85-µm polyacrylate SPME fiber (Supelco) was exposed directly to the solution for 1 h and then retracted into the needle, injected into the GC injector, and exposed at 275°C for 2 min. The phenolics were separated and analyzed by GC–SIM MS. All calibration curves had correlation coefficients of .993 or greater over the concentration range evaluated. Cambridge pads were treated with 7 µg of the standard and 30.5 µg of 2'-hydroxyacetophenone. The pads were extracted with 15 mL HCl–KCl buffer solution with a Burrell wrist action shaker for 1 h. They were allowed to stand at room temperature for 1 h. The aqueous portion was decanted, sampled for 1 h (with stirring) by SPME with an 85-µm polyacrylate fiber (Supelco), and analyzed by GC–SIM MS.

Table II. Recovery of Phenolic Compounds from Cambridge Pad

Phenolic compound	Recovery (%) ± RSD*
Phenol	104.9 ± 6.5
o-Cresol	89.0 ± 7.0
m-Cresol	89.8 ± 8.3
2-Methoxyphenol	106.8 ± 11.4
2,6-Dimethylphenol	86.7 ± 7.5
2,4- & 2,5-Dimethylphenol	109.9 ± 9.57
4-Ethylphenol	107.5 ± 7.44
3-Ethylphenol	109.8 ± 9.0
2,4,6-Trimethylphenol	91.6 ± 2.1
4-Methoxyphenol	99.5 ± 1.9
3-Methoxyphenol	104.4 ± 0.7
Vanillin	122.6 ± 6.7
1-Napthol	47.9 ± 2.3
2-Napthol	68.9 ± 2.7

*RSD = relative standard deviation.

Cigarette smoke condensate analysis

Kentucky reference 2R1F cigarettes from the Tobacco and Health Research Institute were conditioned for 48 h and smoked according to the Federal Trade Commission (FTC) method (19). Cambridge pads were treated with 30.5 µg 2'-hydroxyacetophenone, and the mainstream smoke from two cigarettes was collected. The pads were extracted with 15 mL HCl–KCl buffer solution using a Burrell wrist action shaker for 1 h. They were allowed to stand at room temperature for 1 h. The aqueous portion was decanted, sampled by SPME for 1 h (with stirring) with an 85-µm polyacrylate fiber (Supelco), and analyzed by GC–SIM MS as described.

Results and Discussion

Tomkins and co-workers (10) showed that a 1% acetic acid solution effectively removes the phenolics from a Cambridge pad. This concentration of acetic acid may interfere with the extraction efficiency of the SPME fiber, thus the HCl–KCl buffer described by Pawliszyn (14) was studied. This buffer system has been successfully used for the SPME of chlorinated phenols (14). It was thought that alkylated phenols could be extracted from Cambridge pads efficiently with this buffer and SPME should proceed smoothly as their polarities and pK_a values do not vary as widely as those of the chlorinated phenolics. A methanolic solution containing 14 phenolics of interest was added to a Cambridge pad (7 µg) and extracted with 15 mL of buffer solution. Then, SPME was performed according to the optimized method developed by Pawliszyn. Recoveries near 100%, within relative standard deviation, were obtained for most of the phenolics studied. Recoveries of 1-napthol and 2-napthol were somewhat lower than expected. This result may be due to limited solubility in the buffer solution, which

Table III. Detection Limits for the Analysis of Phenolic Compounds by SPME–GC–SIM MS

Phenolic compound	Amount (nanogram/cigarette)
Phenol	91
o-Cresol	134
m-Cresol	75
2-Methoxyphenol	42
2,6-Dimethylphenol	47
2,4- & 2,5-Dimethylphenol	36
4-Ethylphenol	50
2,4,6-Trimethylphenol	14
4-Methoxyphenol	300
3-Methoxyphenol	187
Vanillin	46
1-Napthol	3.8
2-Napthol	3.8

Table IV. Determination of the Concentration of Phenolic Compounds in Various Cigarettes*

Phenolic	2R1F	Brand A	Brand B	Brand C	Brand D	Brand E
Phenol	9.68 ± 0.57	9.23 ± 2.41	12.37 ± 1.25	10.63 ± 5.90	12.07 ± 1.69	7.77 ± 0.31
o-Cresol	2.90 ± 0.14	2.60 ± 0.73	3.27 ± 0.20	3.33 ± 0.71	3.05 ± 0.35	2.13 ± 0.06
m- & *p*-Cresol	7.08 ± 0.52	6.12 ± 1.80	11.85 ± 5.91	9.28 ± 2.23	7.58 ± 0.90	5.25 ± 0.28
2-Methoxyphenol	0.39 ± 0.02	0.40 ± 0.09	0.43 ± 0.03	0.46 ± 0.03	0.45 ± 0.05	0.35 ± 0.00
2,6-Dimethylphenol	0.44 ± 0.02	ND†	0.43 ± 0.03	0.45 ± 0.09	0.42 ± 0.06	0.30 ± 0.00
2,4- & 2,5-Dimethyphenol	3.07 ± 0.14	2.62 ± 0.72	3.22 ± 0.15	3.27 ± 0.77	2.70 ± 0.26	2.07 ± 0.08
4-Ethylphenol	0.98 ± 0.61	1.52 ± 0.65	1.58 ± 0.47	1.45 ± 0.51	1.90 ± 0.48	1.22 ± 0.03
3-Ethylphenol	2.67 ± 2.48	3.71 ± 4.37	5.48 ± 3.38	5.35 ± 2.77	6.30 ± 4.24	1.23 ± 0.03
2,4,6-Trimethylphenol	0.50 ± 0.05	ND	0.45 ± 0.05	0.50 ± 0.18	0.41 ± 0.06	0.30 ± 0.00
4-Methoxyphenol	0.26 ± 0.11	0.18 ± 0.16	0.23 ± 0.03	0.20 ± 0.10	0.15 ± 0.05	ND
3-Methoxyphenol	0.26 ± 0.06	0.18 ± 0.03	0.23 ± 0.06	0.18 ± 0.10	0.18 ± 0.06	0.15 ± 0.00
Vanillin	0.65 ± 0.14	0.68 ± 0.25	0.58 ± 0.03	0.88 ± 0.25	0.48 ± 0.12	0.35 ± 0.31
1-Napthol	0.37 ± 0.13	0.25 ± 0.1	0.27 ± 0.05	0.33 ± 0.18	0.18 ± 0.08	0.15 ± 0.05
2-Napthol	0.33 ± 0.10	0.25 ± 0.1	0.30 ± 0.05	0.35 ± 0.18	0.21 ± 0.10	0.20 ± 0.05

* Values are reported in micrograms per cigarette.
† ND = not detected.

decreased the extraction efficiency from the Cambridge pad. The results are shown in Table II.

The dynamic range of this method is well within the concentrations of phenolics normally reported in mainstream smoke. All of the phenolic standards were evaluated over a concentration range of 0.1–50 µg and exhibited correlation coefficients of .993 or greater. It was determined that smoke from two cigarettes was sufficient to provide phenolic concentrations within this range. Previously reported methods for the determination of phenolics in cigarette smoke condensate have required a minimum of five cigarettes.

The detection limits for the phenolic compounds were estimated with data from the standard of lowest concentration. Alkyl substitution of the phenolic decreases its solubility in an aqueous matrix. Thus, the phenolic becomes more easily adsorbed by the SPME fiber and detection limits are lowered. For example, phenol was detected at 91 ng per cigarette with a signal-to-noise ratio of 3:1, whereas 2,4,6-trimethylphenol is detected similarly at 14 ng per cigarette. The detection limits are listed in Table III.

Results from the analysis of Kentucky reference 2R1F cigarettes and from five commercial brands are found in Table IV. The Kentucky Reference 2R1F cigarettes underwent six replicate determinations. Commercial cigarettes were analyzed in triplicate. The results are in agreement with previously reported data for phenol, *o*-cresol, and combined *m*- and *p*-cresol. Due to the fact that no derivatization was performed, *m*- and *p*-cresol could not be separated. We were also unable to separate 2,4-dimethylphenol and 2,5-dimethylphenol, thus their concentrations are listed as mixtures. The concentrations for 3-methoxyphenol and 4-methoxyphenol approach the detection limit of the method. The low relative standard deviations for all phenolics exhibit excellent reproducibility for the method. Commercial autosamplers for SPME are now available, thus the method could be easily adapted and provide increased reproducibility.

The polyacrylate SPME fiber is moderately polar, thus nonpolar and very polar analytes are not retained on the fiber. Inferences from nicotine and related weak bases are eliminated by the use of an acidic buffer solution. This property is particularly advantageous when a complex matrix such as tobacco smoke is analyzed because the chromatogram is greatly simplified. Studies using GC–MS in the scan mode indicate simplified spectra with phenolics as the major components. Combination of selected ion monitoring mode with this already selective technique provided spectra with little interference from other components.

Conclusion

The use of SPME–GC–SIM MS has been shown to be a viable method for the determination of the minor phenolics in CSC. The data obtained for Kentucky Reference cigarettes and commercial brands agree with previously reported values. The method has the advantage of minimal sample preparation, excellent selectivity, and environmental suitability. Future applications of this methodology are planned for the evaluation of other mainstream and sidestream cigarette smoke components.

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References

1. E.J. Nanni, M.E. Lovette, R.D. Hicks, K.W. Fowler, and M.F.

Borgerding. Separation and quantitation of phenolic compounds in mainstream cigarette smoke by capillary gas chromatography with mass spectrometry in the selected-ion mode. *J. Chromatogr.* **505:** 365–74 (1990).
2. A.W. Spears. Quantitative determination of phenol in cigarette smoke. *Anal. Chem.* **35:** 320–22 (1963).
3. M.E. Snook, O. T. Chortyk, and R.F. Arrendale. Isolation and identification of the phenolic acids of tobacco smoke. *Tob. Sci.* **29:** 25–31 (1985).
4. A. Prakash and M.S. Ireland. A high performance liquid chromatographic method to quantify phenolic compounds in an isopropanol extract of mainstream cigarette smoke. *Tob. Sci.* **38:** 7–8 (1994).
5. E.L. White, M.S. Uhrig, T.J. Johnson, B.M. Gordon, R.D. Hicks, M.F. Borgerding, W.M. Coleman III, and J.F. Elder Jr. Quantitative determination of selected compounds in a Kentucky 1R4F Reference cigarette smoke by multidimensional gas chromatography and selected ion monitoring-mass spectrometry. *J. Chromatogr. Sci.* **28:** 393–99 (1990).
6. S.S. Hecht, S. Carmella, H. Mori, D. Hoffmann. A study of tobacco carcinogenesis. XX. Role of catechol as a major carcinogen in the weakly acidic fraction of smoke condensate. *J. Natl. Cancer Inst. 66:* 163–69 (1981).
7. C.H. Risner and S.L. Cash. A high performance liquid chromatographic determination of major phenolic compounds in tobacco smoke. *J. Chromatogr. Sci.* **28:** 239–44 (1990).
8. J.D. Mold, M.P. Peyton, R.E. Means, and T.B. Walker. Determination of catechol in cigarette smoke. *Analyst* **91:** 189–94 (1966).
9. G. Jeanty, J. Masse, P. Bercot, and F. Coq. Quantitative analysis of cigarette smoke condensate monophenols by reverse-phase high-performance liquid chromatography. *Beitr. Tabakforsch.* **12:** 245–50 (1984).
10. B.A. Tomkins, R.A. Jenkins, W.H. Griest, R.R. Reagan, and S.K. Holladay. Liquid chromatographic determination of phenols and cresols in total particulate matter of cigarette smoke. *J. Assoc. Off. Anal. Chem.* **67:** 919–23 (1984).
11. B. Gerstenberg and M. Speck. Determination of catechol in cigarette smoke condensate by high-performance liquid chromatography (HPLC) analysis with an automated precolumn sample preparation. *Beitr. Tabakforsch.* **13:** 239–42 (1986).
12. H. Sakuma, M. Kasuma, K. Yamaguchi, and S. Sugawara. The distribution of cigarette smoke components between mainstream and sidestream smoke. *Beitr. Tabakforsch.* **12:** 251–58 (1984).
13. B. Macgillivray, J. Pawliszyn, P. Fowlie, and C. Sagara. Headspace solid-phase microextraction versus purge and trap for the determination of substituted benzene compounds in water. *J. Chromatogr. Sci.* **32:** 317–22 (1994).
14. K. D. Buchholtz and J. Pawliszyn. Optimization of solid-phase microextraction conditions for determination of phenols. *Anal. Chem.* **66:** 160–67 (1994).
15. D.W. Potter and J. Pawliszyn. Detection of substituted benzenes in water at the pg/ml level using solid-phase microextraction and gas chromatography-ion trap mass spectrometry. *J. Chromatogr.* **625:** 247–55 (1992).
16. D.W. Potter and J. Pawliszyn. *Environ. Sci. Technol.* **28:** 298–305 (1994).
17. X. Yang and T. Peppard. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem.* **42:** 1925–30 (1994).
18. W.M. Coleman III. A study of the behavior of Malliard reaction products analyzed by solid-phase microextraction (SPME)/ gas chromatography/ mass selective detection. Presented at the 49th Tobacco Chemists Research Conference, Lexington, KY, September 24–27, 1995.
19. H.C. Pillsbury, C.C. Bright, K.J. O'Conner, and F.W. Irish. Tar and nicotine in cigarette smoke. *J. Assoc. Off. Anal. Chem.* **52:** 458–62 (1969).

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